

Molecular Allergy Diagnosis in Clinical Practice: Frequently Asked Questions

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■ Abstract

Considerable progress has been made in the field of molecular biology in recent years, enabling the study of sensitization to the individual components of an allergenic source, a practice that has been termed molecular allergy diagnosis (MD) or component-resolved diagnosis (CRD). The present review provides the clinician with a practical approach to the use of MD by answering questions frequently asked by physicians on how MD can help improve the diagnosis of allergy in daily clinical practice.

The article is divided into 3 sections. First, we provide a brief review of the importance for the clinician of knowing the main allergens in the different allergenic sources, their structure, and their *in vitro* cross-reactivity before approaching MD (section A). Second, we review the usefulness of MD in clinical practice (section B) and answer frequently asked questions on the subject. Finally, section C addresses the interpretation of MD and its integration with other tools available for the diagnosis of allergy.

Key words: Molecular allergy diagnosis (MD). Component-resolved diagnosis (CRD). Allergen component. Genuine sensitization. Cross-reactivity.

■ Resumen

En las últimas décadas ha habido un gran avance en el campo de la biología molecular permitiendo el estudio de la sensibilización a componentes alérgicos individuales de una fuente alérgica. Dicha práctica se ha denominado Diagnóstico Molecular en alergia (DM) o Diagnóstico por Resolución de Componentes (CRD, según las iniciales en inglés).

El propósito de la presente revisión es ofrecer al clínico un enfoque práctico para el uso del DM respondiendo preguntas frecuentes entre los médicos sobre cómo puede ayudarnos a mejorar el diagnóstico de alergia en nuestra práctica clínica diaria.

La revisión se divide en tres secciones. En primer lugar, se realiza una breve revisión sobre la importancia que tiene para el clínico conocer los principales alérgenos de las diferentes fuentes alérgicas, su estructura y su reactividad cruzada *in vitro* antes de abordar el DM (apartado A). En segundo lugar, está el núcleo de la revisión sobre la utilidad del DM en la práctica clínica (apartado B) respondiendo a las preguntas frecuentes sobre el tema, y, finalmente, se añade un apartado (C) sobre la interpretación e integración del DM con el resto de las herramientas disponibles para el diagnóstico de alergia.

Palabras clave: Diagnóstico molecular en alergia (DM). Diagnóstico por resolución de componentes (CRD). Componente alérgico. Sensibilización genuina. Reactividad cruzada.

Basic Knowledge of Allergens, Cross-reactivity, and Specific IgE Assays

A detailed analysis of individual allergens is beyond the scope of this review. However, before using molecular diagnosis (MD), physicians need to have a basic knowledge of the allergens described to date, their main features, and the commercially available assays for measuring specific IgE (sIgE).

An up-to-date list of allergens and protein families can be found in large allergen databases, including <http://www.allergen.org>, <http://www.allergome.org>, and <http://www.meduniwien.ac.at/allergens/allfam/>.

Table 1 summarizes the main allergens (grouped by protein families) and their common associated clinical features.

Commercially Available Assays for Quantitative Measurement of Specific IgE Against Individual Components

The main commercially available systems in Europe for quantification of individual allergen sIgE include ImmunoCAP (Thermo Fisher Scientific), Immulite (Siemens), and Hytec-288 (Hycor Biomedical).

The complete list of allergens (both native and recombinant) available for each assay can be found at their websites:

<https://www.thermofisher.com/phadia/es/es/product-catalog.html?region=ES>; <https://www.siemens-healthineers.com/es/clinical-specialities/allergy/laboratorian-information>; <https://www.hycorbiomedical.com/noveospecificigeallergens>

Several publications have compared the various assays with each other, showing a relatively good correlation between them, and with clinical diagnosis for the vast majority of allergens. In any case, even though the correlations are good, the measurements obtained with the various assays are not interchangeable [1-6].

In addition to the determination of sIgE against components in singleplex assays, it is possible to determine sIgE to several allergenic components simultaneously. Commercially available multiplex microarrays in Europe include ImmunoCAP ISAC_112i (ThermoFisher Scientific), ALEX2 (MacroArray Diagnostics), and EUROLINE (EUROIMMUN Medizinische Labordiagnostika AG).

ImmunoCAPISAC_112i is an enzyme-linked immunoassay with a fluorochrome-labeled, solid phase secondary antibody of 112 allergenic components from 48 allergen sources. The results are presented in a semiquantitative form (ISU-E); therefore, although they are associated with those obtained with the platforms described below, they are not interchangeable (<https://www.thermofisher.com/phadia/es/es/product-catalog.html?region=ES>).

ALEX2 is a dot-blot (colorimetric) solid phase enzyme-linked immunoassay comprising 117 complete extracts and 178 allergenic components and includes an inhibitor of cross-reactive carbohydrate determinants (CCDs). The results are presented quantitatively by including an IgE curve (kU_A/L), although they are not interchangeable with those of other techniques (<https://www.macroarraydx.com/products/alex>).

EUROLINE is a solid phase, line-blot-type enzyme-linked immunoassay (colorimetric) with various mixtures of complete

extracts and allergenic components adapted to different geographical areas (<https://www.euroimmun.com/products/allergy-diagnostics/>). The results are presented quantitatively in kU_A/L . Once again, this assay is not interchangeable with other techniques.

When to Use Singleplex vs Multiplex Assays

The advantages and limitations of singleplex and multiplex assays have been extensively reviewed [7,8], and a summary of the differences is presented in Table 2. The decision to use singleplex or multiplex testing should take into account various factors:

- a) *Number of allergens to be tested.* If many allergens from a single protein family are involved, multiplex testing might be preferred, especially for those allergen families with limited cross-reactivity, such as seed storage proteins (eg, 2S-albumins, 7S-globulins, and 11S-globulins).
- b) *Purpose of the test. Preferred test sensitivity.* Singleplex testing offers enhanced assay sensitivity and should be the preferred assay when the aim is to determine the extent of sensitization attributable to a given allergenic component, the ratio of sIgE to the component (c-sIgE), and the ratio of sIgE to the whole extract (c-sIgE/we-sIgE) [9], or when the purpose of the study is to monitor sensitization over time.
- c) *Sample volume.* In situations in which the sample volume is limited, such as pediatric patients, multiplex assays could be the approach of choice.
- d) *Availability and costs.* Not all singleplex and multiplex allergen assays are available in every clinical setting or in every country, with the result that it is sometimes not up to the clinician to decide which test to use. However, it is the clinician's responsibility to know the advantages and disadvantages of each technique (Table 2) and how to interpret the results in each specific situation.

As for cost, when more than 12 or 13 individual sIgEs are to be detected, the multiplex assay is thought to be more cost-effective than the singleplex approach and is therefore preferred [10].

Clinical Uses of Molecular Allergy Diagnosis

Allergen Immunotherapy

Why should I perform MD before prescribing allergen immunotherapy?

The general premise before considering allergen immunotherapy (AIT), is to prove that the patient is sensitized to major allergens of the allergenic source. This is one of the main benefits of MD in clinical practice, namely, that it can discern between genuine sensitization to an allergenic source and sensitization resulting from cross-reactivity.

Barber et al [11] recently published an excellent and comprehensive review on the impact of MD on the prescription of AIT and proposed diagnostic algorithms and decision trees driven by component-resolved diagnosis (CRD) for AIT with

Table 1. Main Allergens Families and Associated Clinical Features

Allergen families	Examples	Clinical features
PR-10 or Bet v 1–homologous	Bet v 1. Birch pollen Pru p 1. Peach Cor a 1. Hazelnut and hazelnut pollen Mal d 1. Apple Ara h 8. Peanut Gly m 4. Soy Act d 8. Kiwi Api g 1. Celery Dau c 1. Carrot	High number of sensitizations in the northern area of Spain and central and northern Europe. Present in pollens and plant foods. Sensitization to birch pollen and other pollens from Fagales trees leads to plant food allergy due to extensive cross-reactivity (pollen-food syndrome) in some cases. Heat- and digestion-labile allergens are usually associated with mild reactions such as oral allergy syndrome upon ingestion of fresh plant-derived foods, although anaphylactic reactions have been described in certain circumstances (eg, cofactor-related). Extensive in vitro cross-reactivity.
Profilins	Bet v 2. Birch pollen Ole e 2. Olive Pollen Phl p 12. <i>Phleum pratense</i> pollen (grass) Mer a 1. <i>Mercurialis annua</i> pollen Hev b 8. Latex Pru p 4. Peach Mal d 4. Apple Cuc m 2. Melon	Panallergens that are present in pollens, latex, and plant foods. In Spain, the route of sensitization is usually through sensitization to grass or olive pollen, leading in some cases to mild symptoms upon ingestion of raw vegetables due to cross-reactivity. Allergens are heat- and digestion-labile and, in the case of food allergy, are usually associated with mild reactions, such as oral allergy syndrome, if any, although anaphylactic reactions have been described in certain circumstances (extreme pollen counts). Panallergens usually cause reactions with raw foods. Extensive in vitro cross-reactivity.
nsLTP (nonspecific lipid transfer proteins or PR-14)	Pru p 3. Peach Mal d 3. Apple Jug r 3. Walnut Ara h 9. Peanut Cor a 8. Hazelnut Lac s 1. Lettuce Len c 3. Lentil Tri a 14. Wheat Pla a 3. Plane tree pollen Art v 3. Artemisia pollen Ole e 7. Olive pollen	High number of sensitizations in Spain and the Mediterranean area. The allergens are present in pollens and plant foods. Onset is most frequently via sensitization through the digestive tract, although pollen sensitization has also been described. Allergens resistant to heat and digestion, and, in the case of food allergy, a wide spectrum of symptoms has been described, ranging from asymptomatic and mild reactions to anaphylactic reactions (especially in the presence of cofactors). May cause reactions with both raw and cooked foods. High cross-reactivity both in vitro and in vivo.
TLP (thaumatin like proteins or PR-5)	Act d 2. Kiwi Mus a 4. Banana Pru p 2. Peach Mal d 2. Apple Cor a TLP. Hazelnut Lac s TLP. Lettuce Tri a TLP. Wheat Pla a TLP. Plane tree pollen Cup a 3. <i>Cupressus arizonica</i> pollen	High number of sensitizations in Spain, although there are few commercial methods of measurement. Present in pollens and plant foods. Both pollen and digestive sensitization have been described. Resistant to heat and digestion, and, in the case of food allergy, a wide spectrum of symptoms has been described, ranging from asymptomatic and mild reactions to anaphylactic reactions (especially in the presence of cofactors). May cause reactions with both raw and cooked foods. Intermediate cross-reactivity. Limited in vivo cross-reactivity studies.
Chitinases and other latex-related proteins (PR-3, PR-4, and PR-11)	Hev b 5. Latex Hev b 6. Latex Hev b 7. Latex Hev b 11. Latex Mus a 2. Banana Cas s 5. Chestnut Pers a 1. Avocado Act d chitinase. Kiwi Sola l 1. Tomato Sola t 1. Potato Bra r 2. Mustard Man e 5. Yucca, cassava	High number of sensitizations in Spain and worldwide, although these have been decreasing in recent years. Present in latex and plant foods. Both respiratory and digestive sensitization have been described. Resistant to heat and digestion and, in the case of food allergy, anaphylactic reactions have been described. May cause reactions with both raw and cooked foods. These allergens are associated with the so-called latex fruit syndrome. High cross-reactivity both in vitro and in vivo within each protein family.
Snakin/gibberellin-regulated proteins (GRPs)	Cit s 7. Lemon Pru p 7. Peach Pru av 7. Cherry Pru m 7. Japanese apricot Pun g 7. Pomegranate Cup s 7. <i>Cupressus sempervirens</i> pollen Cry j 7. <i>Cryptomeria japonica</i> pollen	Recently described in Japan and the Mediterranean area (France). Present in pollens (Cupressaceae) and plant foods. Both pollen and digestive sensitization have been described. Resistant to heat and digestion, and, in the case of food allergy, anaphylactic reactions have been described, although the spectrum is very broad. May cause reactions with both raw and cooked foods. Limited in vivo cross-reactivity studies.
Storage proteins of legumes, nuts, seeds, and cereals	Ara h 1, 2, 3, 6. Peanut Jug r 1, 2, 4, 6. Walnut Cor a 9, 14. Hazelnut Ana o 2, 3. Cashew Gly m 5, 6. Soy Ses i 1, 6, 7. Sesame Tri a 19, 20, 21. Wheat	High number of sensitizations in Spain and worldwide, usually initiated in childhood. Present in nuts, seeds, legumes, and cereals (gliadins). Sensitization through the digestive tract, although transcutaneous sensitization associated with atopic dermatitis has also been described. Resistant to heat and digestion, and, in the case of food allergy, frequent anaphylactic reactions have been described, although the spectrum is very broad. May cause reactions with both raw and cooked foods. High in vitro cross-reactivity, although in vivo only cross-reactivity within the same botanical family.

(continued)

Table 1. Main Allergens Families and Associated Clinical Features (continuation)

Allergen families	Examples	Clinical features
Polcalcins (calcium-binding proteins)	Bet v 4. Birch pollen Phl p 7. <i>Phleum pratense</i> (grass) pollen	Polcalcins are panallergens that sensitize a minority of pollen-allergic patients, although their exact prevalence is not well known. Present exclusively in pollen tissue. Their clinical relevance remains controversial. Extensive in vitro cross-reactivity between pollens.
β -Parvoalbumins	Gad c 1. Cod Cyp c 1. Carp	High number of sensitizations in Spain and worldwide, with onset usually in childhood. Main allergens in fish. May produce symptoms through both the digestive and respiratory routes. Heat- and digestion-stable proteins can cause allergic symptoms with both raw and cooked foods. High cross-reactivity both in vitro and in vivo between practically all fish. Patients can tolerate fish low in β -parvalbumin, such as tuna or swordfish (<i>Xiphias gladius</i>).
Tropomyosins	Pen a 1. Prawn Der p 10. Mites (<i>Dermatophagoides pteronyssinus</i>) Bla g 7. Cockroach (<i>Blattella germanica</i>) Ani s 3. <i>Anisakis simplex</i> Myt e 1. Mussel Mer ly 1. Clam Sep l 1. Cuttlefish Oct l 1. Octopus	High number of sensitizations in Spain and worldwide, with onset usually in childhood. Main allergens in shellfish. May induce symptoms through both the digestive and respiratory routes. Heat- and digestion-stable proteins can cause allergic symptoms with both raw and cooked foods. Extensive cross-reactivity both in vitro and in vivo between crustaceans, cephalopods, and mollusks. In vitro cross-reactivity also reported with nematodes and insects.
Serum albumins	Fel d 2. Cat Can f 3. Dog Bos d 6. Veal Sus s 1. Pig Equ c 3. Horse Gal d 5. Chicken, hen egg	Proteins present in various biological and solid fluids, eg, cow's milk, mammalian meats (beef and pork), and poultry (chicken). Sensitization to serum albumin can cause respiratory symptoms due to sensitization to dander/mammalian epithelium, for example, in cat-pig syndrome or against egg/chicken and feather meats such as bird-egg syndrome, as well as reactions against foods such as mammal meat (eg, veal) and milk.
Lipocalins	Can f 1, 2, 4, 6. Dog Fel d 4. Cat Equ c 1. Horse Mus m 1. Mouse	Mammalian-derived major allergens causing respiratory sensitization. Associated with greater severity of respiratory symptoms (asthma). Stable proteins. Major allergens in pet allergy. Limited cross-reactivity between different species.
CCD and "CCD-bearing proteins"	Ana c 2. Pineapple MUXF3. (fraction Ana c 2 from pineapple) Jug r 2. Walnut Pla a 2. Plane tree pollen Cup a 1. <i>Cupressus arizonica</i> pollen Cry j 1. <i>Cryptomeria japonica</i> pollen Phl p 1, 4. Pollen of <i>Phleum pratense</i> (grass) Cyn d 1. Pollen of <i>Cynodon dactylon</i> (grass) Ole e 1. Olive pollen	May be present in approximately 30% of polysensitized patients. Marker for sensitization to cross-reactive carbohydrate residues of proteins (pollen, hymenoptera, latex). Rarely associated with clinical symptoms. Associated with multiple positive results, especially in vitro specific IgE to whole extracts. May be detected when using purified native allergens (MUXF3, nJug r 2, nPla a 2, nCup a 1, nCry j 1, nPhl p 4) but not when using recombinant allergens without CCDs (rOle e 1 and rPhl p 1) or CCD inhibitors.

pollens, epithelia, and some foods (nonspecific lipid transfer protein [nsLTP] containing vegetables and peanuts).

Which allergens should be included in a pollen allergy study when considering AIT?

The panel of allergens should be chosen depending on the area and the availability of the components. The extended panel would include markers of genuine sensitization to pollen (Phl p 1/5, Ole e 1, Par j 2, Cup a 1, Art v 1, Sal k 1, Pla a 1/2, Amb a 1, Pla l 1, Bet v 1), as well as, in areas of high olive pollen exposure, Ole e 7 and Ole e 9. Sensitization to cross-reactive allergens, namely, Bet v 2, Phl p 12, Hev b 8, Mer a 1 (profilins), and Phl p 7 or Bet v 4 (polcalcins) should also be studied.

In general, when the sensitization is clinically relevant and the patient is sensitized mainly to the major allergens of the pollen source, AIT should be prescribed, irrespective of the

sensitization to cross-reactive allergens. The general premise is that the AIT product should be quantified and standardized for these major allergens.

If the patient is only or mainly sensitized to cross-reactive or minor allergens, AIT should not be prescribed, since the content for minor allergens in AIT preparations is unknown and variable and there is no evidence of the efficacy of AIT products in patients sensitized only to minor allergens.

Which allergens should be included in a house dust mite allergy study when considering AIT?

CRD using purified and/or recombinant allergens can improve the accuracy of specific IgE testing in house dust mite (HDM) allergy, although availability is limited worldwide. The WHO/IUIS allergen nomenclature subcommittee currently includes up to 31 *Dermatophagoides pteronyssinus* and

Table 2. Comparison of a Representative Singleplex (ImmunoCAP) and Multiplex (ISAC) IgE Testing Assay

	Singleplex (ImmunoCAP)	Multiplex (ISAC)
Amount of allergen on assay	~1-2 µg/ determination	~100 pg/spot
Read-out	Quantitative	Semiquantitative
Amount of serum needed	~40 µL/ determination	~30 µL/chip (112 allergens)
Procedure	Automated	Manual
Result variation coefficient	Low	Medium
Interference with IgG4	No	Yes
Interference in cases with high total IgE levels	Yes	No
IgE detection (affinity)	Low and IgE high affinity	High affinity IgE
Useful for patient monitoring and follow-up	Yes	No
CCD-inhibitor added	No	No
Results units	kU _A /L	ISU-E
Global availability	Yes	No

Dermatophagoides farinae allergens, as well as 13 allergens from *Blomia tropicalis* and other allergens from storage mite species. The *Dermatophagoides* species group 1, group 2, and group 23 allergens are the immunodominant allergens. The group 4, 5, 7, and 21 allergens exhibit mid-tier allergenicity, and the other groups minor or unknown allergenicity [12].

As with the indication of AIT for pollen allergy, when sensitization to HDM is clinically relevant in respiratory allergy and the patient is sensitized mainly to the major allergens from group 1 (Der p 1 or Der f 1) and/or group 2 (Der p 2 or Der f 2), AIT should be prescribed, irrespective of sensitization to other allergens [13]. The general premise is that the commercial AIT product should be quantified and standardized for these major allergens.

Predominant sensitization or monosensitization to the major allergen Der p 23 is of particular interest. This allergen is present in commercial AIT extracts, although to date, only group 1 and 2 HDM allergens are quantified and standardized in these extracts; therefore, we need more evidence to recommend HDM AIT in this case.

Which allergens should be included in a pet epithelia allergy study when considering AIT?

Regarding cat allergy, Fel d 1 is a major allergen, with sensitization rates of up to 92% of cat-allergic patients, as reported in a recent study on the efficacy of cat and dog AIT [14]. Thus, despite substantial differences in Fel d 1 content among different immunotherapy extracts, it seems reasonable to confirm sIgE sensitization to Fel d 1 before prescribing AIT for cat allergy.

Regarding dog allergy, the pattern of sensitization is more heterogeneous, and wide variability in allergen content has

been documented between commercial extracts [15], thus potentially explaining the poor and conflicting results for clinical efficacy of dog AIT in the medical literature [11,16]. To date, dog allergens available for determination of sIgE include Can f 1, Can f 2, Can f 3, Can f 4, Can f 5, and Can f 6.

Positive sIgE to Can f 3 or Can f 6 in the absence of sensitization to other dog allergens suggests cross-reactivity due to primary sensitization to other epithelia, with the result that AIT may not be advisable [11].

In cases of monosensitization to Can f 5 (reported in up to 37% of dog-allergic patients [17]), it was recently reported that children react differently to male and female dog extract in conjunctival provocation tests, suggesting tolerance to female dogs [18]. Therefore, in cases of monosensitization to Can f 5, it may be better to prescribe male dog avoidance rather than to prescribe an AIT with unknown Can f 5 content.

Which allergens should be included when considering AIT for allergy to *Alternaria*?

sIgE to Alt a 1 should be assessed before considering the prescription of AIT in a patient with clinically relevant sensitization to *Alternaria*. Alt a 1 is a major allergen, recognized by more than 90% of *Alternaria*-allergic patients, and a marker of primary sensitization to this fungus. A recent clinical trial showed the efficacy and safety of AIT with a commercial extract of Alt a 1 [19].

What allergen profile should I request before prescribing hymenoptera venom immunotherapy?

Before prescribing venom immunotherapy it is advisable to determine the levels of serum tryptase, total IgE (tIgE), and sIgE to the whole extract of all hymenoptera venoms relevant in the specific area (eg, bee, common wasp or yellow jacket, paper wasp, Mediterranean or European paper wasp, European hornet, Asian wasp) and sIgE to CCDs (MUXF3). The ratio between whole extract sIgE and tIgE (if detected using the same technique) informs us of the relevance of this allergenic source in the individual patient's venom allergy and is especially important in cases of low levels of tIgE [9].

After confirmation of in vitro sensitization to the whole venom extract and exclusion of sensitization to a CCD, a study of sensitization to individual components should be carried out. In the case of bee venom allergy, sIgE to Api m 1, 2, 3, 5, and 10 (and Api m 4 if available) should be determined; in vespid venom allergy, sIgE to Ves v 1, Ves v 5, and Pol d 5 (and Pol d 1 if available) should be determined [20].

The ratio between the allergen component sIgE and whole extract sIgE (c-sIgE/we-sIgE) allows us to evaluate the extent of sensitization attributable to a given allergic component [9].

If genuine sensitization cannot be identified with these allergen profiles, as addressed in the following question, the use of other techniques, such as CAP inhibition, may be a useful strategy [21].

In cases of double or multiple positivity to hymenoptera venom, can MD help me to determine the genuine sensitizer?

MD can prove useful for distinguishing between genuine sensitization and cross-reactivity only in cases with double or multiple sensitizations to hymenoptera venoms.

The current panel of commercially available bee venom allergens considered as markers of genuine sensitization includes Api m 1, Api m 3, and Api m 10. Api m 4 is not commercially available in Spain. These allergens can also be markers of bumble bee venom allergy [11,20].

The hyaluronidase Api m 2 is a potential marker of bee venom allergy but shows limited cross-reactivity with Ves v 2 and Pol d 2 in the absence of CCDs [11,20].

Ves v 5 and Pol d 5 show high in vitro cross-reactivity. Commercially available whole extracts from vespid venom (common wasp or yellow jacket, paper wasp, and Mediterranean or European paper wasp) are supplemented with antigen 5 and also present high in vitro cross-reactivity.

Api m 5, Ves v 3, and Pol d 3 belong to the dipeptidyl peptidase-IV family and have high in vitro cross-reactivity that prevents their use as markers of genuine sensitization. In fact, Api m 5 can be a marker of vespid venom allergy [11,20].

The phospholipase A1 allergens Ves v 1 and Pol d 1 also present high in vitro cross-reactivity, which prevents their use as markers of specific vespid venom allergy [11,20].

Sensitization to CCD should be ruled out when multiple in vitro sensitizations to whole hymenoptera venom extracts (especially bee venom and common wasp venom and less frequently to paper wasp venom). The interference of CCDs can be minimized by preincubation of the serum with a CCD inhibitor.

Are some sensitization profiles associated with a higher risk of adverse effects during AIT?

Some sensitization profiles are associated with a risk of adverse effects induced by grass and olive pollen subcutaneous immunotherapy (SCIT).

Sastre et al [22] reported a significant association between the number of grass allergens (Phl p 1, Phl p 5, and Phl p12) that sensitized patients and the total number of local and systemic adverse events with grass pollen SCIT.

In a trial on the safety and efficacy profile of a grass sublingual immunotherapy tablet, the incidence of adverse events was correlated with the highest sIgE levels for Phl p 5 or Phl p 1 [23].

Sensitization to Ole e 7 (an olive pollen nsLTP) has been associated with severe clinical symptoms and systemic adverse reactions with AIT in regions with high levels of olive pollen exposure. In a recent algorithm to support the selection of olive pollen AIT, Barber et al [24] recommend avoiding prescription of AIT in patients sensitized to Ole e 7, irrespective of sensitization to Ole e 1.

Regarding markers of adverse reactions to immunotherapy in HDM allergy, in their retrospective post hoc analysis to evaluate whether the sensitization profile of HDM was associated with the efficacy and safety of HDM SCIT, Gadermaier et al [25] reported an association between sensitization to Lep d 2 and a higher rate of systemic reactions during treatment. However, these results need to be validated in prospective studies.

No specific sensitization profile to individual components has been associated with the safety of SCIT with cat and dog extracts in 2 recent studies by Uriarte et al [26,27] using an ultrarush up-dosing phase protocol.

Which hymenoptera allergens are associated with therapeutic failure or risk of adverse effects?

Api m 10 accounts for a small percentage of bee venom (less than 1% of dry weight) and an apparently unstable nature not only as native Api m 10, but also as a recombinant allergen.

Patients with predominant sensitization to Api m 10 may be at risk of therapeutic failure, possibly owing to its underrepresentation in some venom immunotherapy preparations, because these commercial treatment extracts lack the allergen [28].

The risk of adverse events during bee venom immunotherapy in a Spanish population of bee venom-allergic patients has been associated with the presence of sIgE to Api m 4, especially at levels >0.98 kU/L [29,30].

Will a CRD-driven prescription of AIT predict better efficacy of AIT?

Various studies point towards lower efficacy of AIT in cases where recognition of molecular spreading is more complex. The use of allergenic molecules in various clinical studies [31-33] aimed at monitoring changes in the specific antibody repertoire of patients receiving AIT has proven successful.

Specific studies designed to address the efficacy of MD-driven AIT are necessary, since, to date, only post hoc analyses have been performed, with inconsistent results. While Chen et al [32] suggest that molecular assays constitute a promising approach for predicting and monitoring the efficacy of AIT for HDM allergy, Arroabarren et al [33] could not find a significant association between efficacy of AIT and the HDM sensitization profile.

A recent study by Rodríguez-Domínguez et al [13] on 24 HDM-allergic patients who had received 1 year of SCIT for HDM allergy (Alutard SQ) concluded that the stratification of patients according to molecular sensitization profiles and molecular monitoring of AIT-induced IgG responses may enhance the success of AIT.

These recent studies emphasize that molecular assays constitute a promising approach for predicting and monitoring the efficacy of AIT. However, prospective studies are needed to confirm that certain molecular IgE sensitization profiles are predictive biomarkers of the efficacy of AIT.

The clinician needs to be aware that factors other than the sensitization profile account for the efficacy of AIT, such as appropriate dose, duration of treatment, and adherence.

Once the patient's sensitization profile is known, can I choose an AIT extract accordingly?

The heterogeneity of the AIT preparation has been conclusively demonstrated for several allergen sources, including birch and grass pollen, HDM, and insect venom preparations [34-37]. Therefore, the recommendation is to use commercial AIT extracts with quantified and standardized major allergens and evidence of efficacy.

Polysensitization/Complex Patient

How can MD improve the diagnosis of polysensitized/complex patients?

When a patient is sensitized to both food and respiratory allergens, there are 2 possible scenarios: either the patient is

genuinely sensitized to both food and respiratory allergens, or the food allergy is caused by cross-reactivity due to primary sensitization to the inhalant allergen. The only way to differentiate one situation from the other is to perform MD after taking an extensive clinical history and detecting sensitization to whole allergen extracts.

In this scenario, the MD study serves to rule out pollen-food syndrome, to help stratify the risk of severe reaction in case of food allergy, to guide food challenges, and to advise on food avoidance.

Which allergens should be included in the study of pollen-food syndromes?

Pollen-food syndrome (PFS) involves a reaction to foods when the primary sensitization to the allergen has occurred through the respiratory route.

The main allergen families related to PFS are Bet v 1 homologs (PR-10) and profilins [7]. Both protein families show extensive cross-reactivity in which a single marker (Bet v 1 for PR-10 and Bet v 2 or Phl p 12 for profilins) may be sufficient to define sensitization to the whole allergen family.

Further IgE testing with food allergens belonging to the same family would potentially create many positive results with questionable clinical relevance.

PFS due to nsLTP cross-reactivity has also been described, mainly for Art v 3, Pla a 3, and Pru p 3 [38,39], although recent reports [40] also indicate cross-reactivity between Ole e 7 and Pru p 3, thus explaining how Ole e 7 could play a new role as a primary sensitizer, producing sensitization to peach nsLTP in regions with high olive pollen exposure. All these pollen LTPs are available for determination of sIgE in both singleplex and multiplex assays. In PFS due to cannabis LTP (Can s 3), cross-reactivity to multiple food-containing LTPs is well established. However, its diagnosis relies on the determination of sIgE to Can s 3, which is not yet commercially available [41].

Thaumatococcus-like proteins (TLPs) are also responsible for PFS. Present in allergenic sources such as cypress, plane tree, *Artemisia* pollen, and cannabis, TLPs cause cross-reactivity with fruits including Rosaceae, banana, kiwi, grape, melon, and almond [42]. The limited number of TLPs available for MD is still the main problem when studying this cause of PFS, since they are not available in singleplex assays and only Act d 2 (ImmunoCAP ISAC) and Mal d 2 (ALEX2) are available in multiplex assays.

Snakin/gibberellin-regulated proteins (GRPs) were recently recognized as being responsible for PFS among cypress pollen and fruits (mainly peach, citrus fruits, and pomegranate) [43]. Only Pru p 7 is currently available in the singleplex version of the ImmunoCAP assay, although it is very likely that Cup a 7 will soon be commercially available.

Other allergen families responsible for less prevalent PFS include β -1,3 glucanase polygalacturonase and isoflavone reductase [7]. These should be assessed individually according to the culprit allergenic sources.

Regarding latex-fruit syndrome, several allergen families have been involved, including class I chitinases (Hev b 6, 11, and 14), β 1,3 glucanases (Hev b 2), patatin-like proteins (Hev b 7), nsLTP (Hev b 12), and acidic protein (Hev b 5). Singleplex sIgE assays are available for some of these latex allergens [44].

Which allergens should be included in the study of respiratory and meat cross-reactivity syndromes?

Serum albumins are also responsible for respiratory and meat cross-reactivity syndromes, including pork-cat and bird-egg syndromes. Since mammalian serum albumins are highly cross-reactive, determining sIgE to Can f 3, Fel d 2, Bos d 6, and Sus s 1 alone may be sufficient and should be guided by the suspected primary allergenic source according to the clinical history. When the clinical history suggests bird-egg allergy syndrome, sIgE to Gal d 5 should be specifically determined, since homology to mammalian albumins is very low [7].

How should we interpret sensitization to CCDs?

The first description of the presence of IgE to CCDs was made in 1981 by Aalberse et al [45]. It is now clear that anti-CCD IgE has little or no clinical relevance but is a confounder for in vitro diagnosis [46].

sIgE to CCD-bearing proteins mainly recognize a core of the amino sugar (α 1,3 fucose) linked to N-acetylglucosamine (N-glycan). The main related structures are MUXF and MMXF glycans.

IgE to CCDs result in broad in vitro cross-reactivity, especially among pollen, plant food, latex, and hymenoptera venom. The overall prevalence of sIgE to CCDs is about 25%, reaching 71% in patients with multiple pollen sensitizations. Given the low clinical relevance of sIgE to CCDs, their interference can be overcome by 2 strategies: one is to include a marker of CCD (MUXF3) in screening allergy panels as an alert signal, and the second is to add a CCD inhibitor to the detection method. A potential disadvantage of the latter approach is that it decreases test sensitivity [47].

α -Gal (α 1,3 galactose) also belongs to the N-glycan family, although in contrast to typical CCDs, it has been associated with severe allergic reactions to meat [46].

Risk Stratification in Food Allergy

CRD can improve diagnostic accuracy and help stratify clinical risk; however, results must always be interpreted within the context of the patient's clinical history.

The classic concept that remains valid is that sensitization to allergenic proteins with greater thermal stability and resistance to proteolysis and enzymatic digestion (storage proteins, nsLTPs, gliadins, thaumatococcus-like proteins, GRPs, tropomyosins, parvalbumins, caseins, and ovomucoid) is associated with a higher risk of systemic or severe reactions, while sensitization to acid- and heat-labile proteins (profilins or PR-10) is generally associated with mild symptoms and may not even be clinically relevant [48].

However, we would like to emphasize that there are exceptions to this rule, and severe reactions have been reported after ingestion of plant-derived foods in patients sensitized to PR-10 proteins or profilins [49,50] who have been exposed to high doses of allergen in the presence of cofactors. Moreover, sensitization to stable allergens such as nsLTPs is characterized by very heterogeneous clinical expression and is more frequently related to mild symptoms in the absence of cofactors [51].

Can sIgE to “markers of severity” predict clinical reactivity?

Detection of specific IgE in serum is strictly a marker of allergic sensitization. The presence of IgE alone cannot predict the probability of an allergic reaction.

Many variables contribute to the clinical expression of sensitization. Some are related to the allergen itself (such as the concentration of the protein in the edible food, the degree of homology, and stability to heat/digestion), whereas others are related to the immune response (IgE antibody concentration, specificity, affinity, and effector cell reactivity) and host-dependent factors including cofactors (exercise, alcohol, intake of nonsteroidal anti-inflammatory drugs, illness).

Therefore, the clinical relevance of allergic sensitization to an allergen molecule will always have to be interpreted in the context of the clinical history and a controlled oral food challenge (OFC) when needed.

Which allergens present potential clinical cross-reactivity?

Molecule-based sensitization tests may reveal the degree and potential clinical relevance of further cross-reactivity to related molecules of a protein family.

In the case of protein families with highly cross-reactive allergens (eg, Bet v 1 homologs, profilins, nsLTPs, polcalcins [calcium-binding proteins], serum albumins, grass pollen major group 1 and 5 allergens, parvalbumins, and tropomyosins), it is sufficient to test only 1 member of the family and then conduct a thorough clinical work-up to identify relevant clinical cross-reactions.

In the case of allergens of limited cross-reactivity (seed storage proteins such as 2S-albumins, 7S-globulins [vicilins], and 11S-globulins [legumins], as well as lipocalin subfamilies), an appropriate panel of related allergens (from the same protein family) could be used to demonstrate or exclude subsequent (serological) cross-reactivity. Usually, the allergen with the highest sIgE level will be the primary sensitizer. In the case of nut allergy in patients sensitized to seed storage proteins, the highest clinical reactivity is related to the botanical family, with strong correlations between cashew-pistachio, walnut-pecan, and walnut-pecan-hazelnut-macadamia [52,53].

However, it is again important to underscore that serological cross-reactivity is not equivalent to clinical cross-reactivity, as rates of sensitization significantly outnumber clinical allergy. Although molecular diagnosis may be helpful in assessing the possibility of clinical cross-reactivity, the correlation with the clinical history and OFC remains the gold standard.

Can sensitization to some allergens be used to guide challenge tests?

Using the sensitization profile can help characterize the patient's risk of a severe reaction and determine who should undergo an OFC, although, to date, there are no well-defined cut-offs for most allergens.

The diagnostic value of CRD for predicting a positive OFC has been established mainly for peanut and tree nuts.

Ara h 2 is the most important predictor of symptomatic peanut allergy, and several recommended sIgE Ara h 2 cut-

offs have been proposed, with important variations between populations [54-56].

Cor a 9 and Cor a 14 have been associated with systemic reactions to hazelnut. Various diagnostic cutoffs have been proposed for both allergens [52,57].

Levels of sIgE to Jug r 1 or 4 ≥ 0.35 kU_A/L have been reported to provide the best diagnostic method for identifying walnut-allergic patients [58].

Cut-off points have also been defined for predicting a positive OFC for cashew (Ana o 3), soy (Gly m 8) and wheat (Tri a 19) (reviewed by Foong et al [59]).

Regarding milk and egg allergy, CRD has not consistently been shown to predict baked milk or baked egg tolerance.

Can sensitization to some allergens be used to guide avoidance measures?

When the MD study yields a positive result to cross-reactive food allergens, the most suitable approach is not to indiscriminately remove tolerated foods from the diet merely because they are related to an allergen that caused a reaction.

Some important aspects of clinical cross-reactivity that may assist when deciding to undertake the OFC or expand a diet were recently addressed by Cox et al [60].

Component-Resolved Diagnosis in Idiopathic Anaphylaxis

Is MD useful in the study of idiopathic anaphylaxis?

Idiopathic anaphylaxis is a diagnosis of exclusion when all specific possible triggers of recurrent anaphylaxis have been ruled out based on a standard step-up allergy diagnosis.

MD has proven useful for identifying causes of anaphylaxis previously labeled idiopathic. In the case of singleplex assays, the clinician may choose the single components to be tested, as, for example, in the case of delayed meat anaphylaxis, which can be diagnosed by detecting sIgE to α -gal (galactose- α -1,3-galactose), to ω -5-gliadin (Tri a 19) (in cases of suspected wheat-dependent exercise-induced anaphylaxis), or to other known allergens [61].

Another approach is the use of multiplex assays, in which a high number of individual allergen molecules are tested simultaneously. MD arrays enable this approach to be used as a screening tool to assess sensitization by identifying potential triggers of anaphylaxis [61].

Importantly, in the diagnosis of idiopathic anaphylaxis, it is necessary to carefully assess possible concurrent conditions such as cofactors (eg, nonsteroidal anti-inflammatory drugs, exercise, alcohol) and potential mast cell disorders.

Which allergens are not well represented in commercial whole extracts for prick testing or determination of sIgE and should therefore be assessed in the anaphylaxis work-up?

Not all allergens are well represented in commercial assays; allergists must know which allergens are underrepresented in their clinical practice. For example, many years ago, the latex allergen Hev b 5 and vespid antigens (Ves v 5 and Pol d 5) were poorly represented in commercial whole extracts. The manufacturers have resolved this issue by supplementing

whole extracts with relevant allergens such as whole latex extract with Hev b 5 or whole vespid extracts with Ves v 5 or Pol d 5. A similar situation has been observed for ω -5-gliadin (Tri a 19) in whole wheat extracts, although the issue has not yet been resolved.

Similar observations can be made for allergen extracts used for in vivo and in vitro diagnostic tests that do not contain lipophilic allergens such as oleosins, because these proteins are lipophilic and nearly insoluble in aqueous solutions. Given that oleosins of sesame, peanut, and hazelnut have been shown to be responsible for anaphylaxis, a negative sIgE result with whole extract does not rule out the implication of these specific foods.

What Does Molecular Allergy Diagnosis Provide in Occupational Allergy?

sIgE reactivity to occupational allergen components has been poorly investigated, with the notable exception of latex allergy and cereal flour responsible for baker’s asthma. For other occupational allergens, it remains necessary to evaluate the relevance of single allergen molecules to assess the sensitization induced by occupational exposure [62].

Twenty-seven wheat allergens are listed in the WHO/IUIS allergen nomenclature database, although only a few are commercially available for testing individually. Tri a 19 (α -5-gliadin) is not relevant for the diagnosis of baker’s

asthma but is involved in wheat-dependent, exercise-induced anaphylaxis and in early childhood type I wheat allergy. In Spanish bakers, the wheat nsLTP Tri a 14 was described as a major allergen in baker’s asthma [62].

In the 1980s, latex allergy was epidemic in places where powdered natural rubber latex gloves were used, with exposed health care workers and some patients (eg, those with spina bifida) being affected. The 2 main problems in latex allergy are (a) systemic type I reactions (anaphylactic shock in anesthetized patients was the most frequent) during medical/surgical procedures due to mucosal or parenteral release of allergens and (b) latex-fruit syndrome due to cross-reactivity with vegetables.

A systemic type I reaction can occur after sensitization to any latex allergen except isolated Hev b 8 (profilin). There are at least 15 latex allergens. Hev b 1 to Hev b 15 belong to different allergen families [44], but only Hev b 1, Hev b 3, Hev b 5, Hev b 6, Hev b 8, and Hev b 11 are commercially available

Patients who have latex allergy and require surgery or other specialized procedures in operating/procedure rooms should be the first case of the day and in a latex-free room. Patients with isolated sensitization to Hev b 8 (profilin) [63] and/or CCDs and positive sIgE to latex whole extract with negative results for other latex allergens do not need to avoid latex in surgical procedures [64].

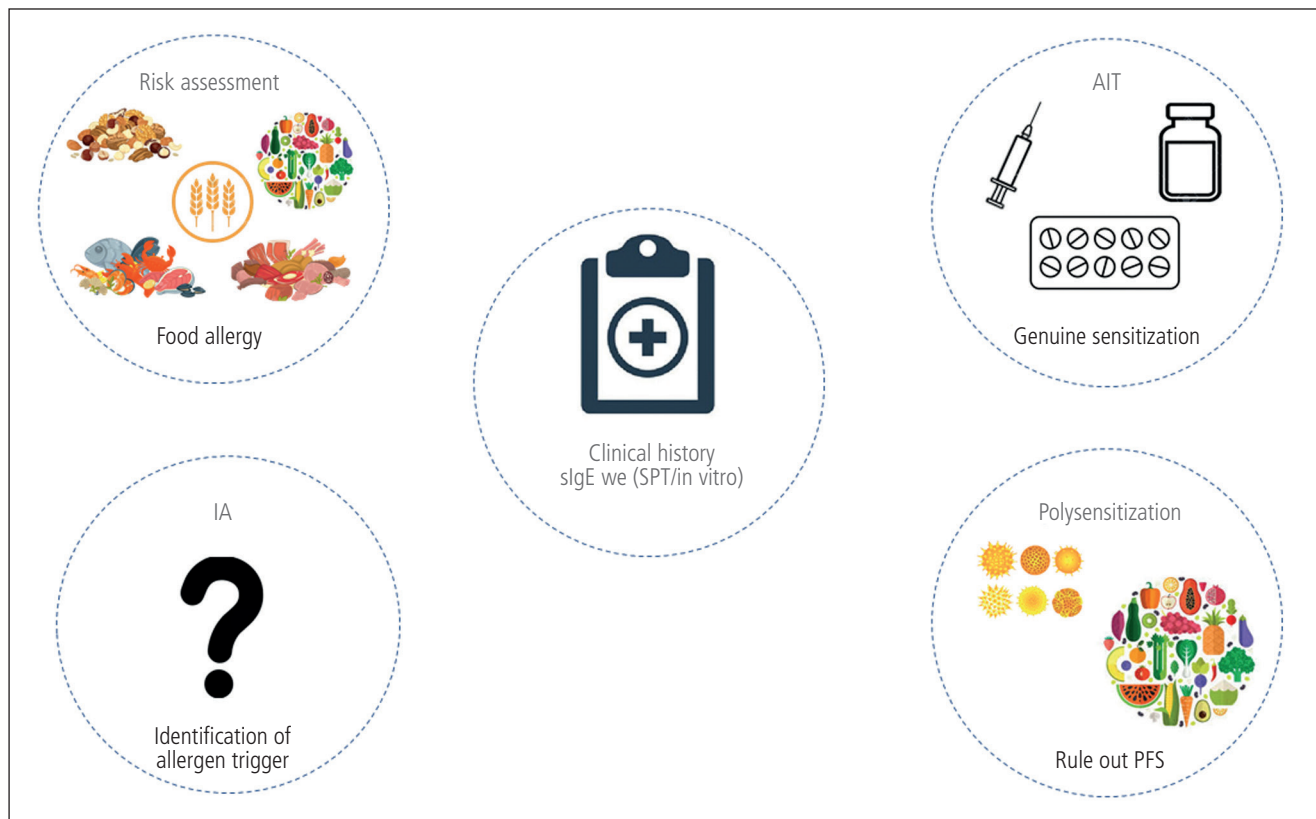


Figure. Main clinical uses of molecular diagnosis of allergy. IA indicates idiopathic anaphylaxis; AIT, allergen immunotherapy; sIgE we, specific IgE whole extract; SPT, skin prick test; PFS, pollen-food syndrome.

Interpretation of Molecular Allergy Diagnosis

Is the generation of an extensive IgE sensitization profile a disadvantage of multiplex assays?

One of the main criticisms leveled at MD by multiplex assay is the detection of unexpected sensitizations that may confuse the clinician when interpreting the results.

However, this is not an uncommon situation in routine clinical practice, where skin tests are often performed with extensive panels of whole allergenic extracts, sometimes yielding unexpected results. Therefore, it seems reasonable to interpret these results in the same way as with other clinically irrelevant sensitizations to food or respiratory allergens, namely, by taking a meticulous clinical history to assess the clinical relevance of the sensitization and performing controlled challenges when needed.

The detection of silent sensitivities may give the clinician the chance to investigate other hypersensitivities and to alert the patient to possible risks; however, sensitization itself (without a concordant clinical history or positive challenge test result) should not drive avoidance measures.

Considering all the benefits of MD in clinical practice, can I skip conventional allergy diagnostic tests?

It may be tempting to test only the levels of sIgE to the major allergens from the suspected allergenic source when we know the most common sensitization profile in our area. However, this practice could lead to misinterpretation of the results.

Recently, Pascal et al [9] proposed the use of ratios of the sIgE of a given specific allergen component to the levels of sIgE to its whole extract (c-sIgE/we-sIgE) to evaluate the extent of sensitization attributable to this specific allergic component.

Furthermore, the number of allergen components available for diagnostics remains limited, and detecting sensitization to certain allergenic sources is only possible using whole allergen extracts.

The clinical history continues to be the traditional approach and should never be replaced by diagnostic tests, which—importantly—only detect sensitization. The allergy work-up should always be based on a meticulous clinical history aimed at identifying the culprit allergen and the clinical relevance of the sensitizations detected.

The main clinical uses of MD are summarized in the Figure.

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